Placental evolution from a three-dimensional and multiscale structural perspective

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ABSTRACT

The placenta mediates physiological exchange between the mother and fetus. In placental mammals, all placentas are descended from a single common ancestor and functions are conserved across species; however, the placenta exhibits radical structural diversity. The selective pressures behind this structural diversity are poorly understood. Traditionally, placental structures have largely been investigated by grouping them into qualitative categories. Assessing the placenta on this basis could be problematic when inferring the relative 'efficiency' of a placental configuration to transfer nutrients from mother to fetus. We argue that only by considering placentas as 3D biological structures, integrated across scales, can the evolutionary questions behind their enormous structural diversity be quantitatively determined. We review the current state of placental evolution from a structural perspective, detail where 3D imaging and computational modelling have been used to gain insight into placental function, and outline an experimental roadmap to answer evolutionary questions from a multiscale 3D structural perspective. Our approach aims to shed light on placental evolution, and can be transferred to evolutionary investigations in any organ system.

KEYWORDS: placenta, mammal, three-dimensional, microscopy, evolution

1. INTRODUCTION

The placenta mediates the exchange of nutrients between the mother and fetus and is integral to healthy reproduction in placental mammals. Arguably no organ displays more structural diversity in placental mammals than the placenta, despite having conserved functions and a single evolutionary origin (Wildman et al., 2006, Griffith and Wagner, 2017). The evolutionary drivers and functional consequences of this diversity are poorly understood, although it has been proposed that the evolution of placental structural diversity may be coupled to different reproductive strategies (Capellini et al., 2011), environmental pressures (Capellini et al., 2015), and interparental conflict (Kazemian et al., 2019). Determining why this structural diversity exists would reveal fundamental insights into the factors determining reproductive success.

A large body of work on placental mammals has described placental structure using a qualitative schema of types derived from two-dimensional (2D) histology sectioning. Studies employing this schema have contributed greatly to our understanding of comparative placentation; however this schema may have limitations if it overlooks structures that can only be understood in three dimensions (3D) and does not treat the placenta as an integrated multiscale system. Placental types in some species are assumed to permit more 'efficient' nutrient transfer from mother to fetus than others. This is a central assumption in investigations of placental evolution, but it lacks empirical validation. The advent of multiscale imaging coupled to physiological modelling now provides an opportunity to test and develop these ideas in a quantitative framework encompassing the full range of physiologically relevant scales.

Here, we review the prevailing structural understanding of, and outstanding questions in, the evolution of the placenta in placental mammals. We examine recent progress in applying 3D imaging techniques to placentas in placental mammals. We argue that ultrastructural and multiscale data, coupled with physiological modelling, must be integrated into an understanding of the placenta as a whole if assumptions about efficiency are to be supported and evolutionary hypotheses tested. We lay out an experimental roadmap to do this. Although we focus on placentas, the approaches outlined here are transferrable to the evolution of any organ system. We conclude by

briefly exploring how our integrated structural perspective can be applied to address questions in evolutionary biology more broadly.

2. THE PLACENTA OF PLACENTAL MAMMALS

A placenta can be defined as any 'apposition or fusion of the fetal membranes to the uterine mucosa for physiological exchange' (Mossman, 1937), producing a fetally-derived extracorporeal organ separating fetal and maternal circulatory systems (Steven, 1975, Burton, 2022) (Fig. 1A). The function of the placenta is to sustain fetal development (Fig. 1B) by facilitating the transfer of nutrients and oxygen to the fetus from maternal blood and removing waste from fetal blood. The placenta also acts as a barrier against toxins, pathogens, and maternal hormones, and mediates maternal-fetal communication by secreting a wide array of hormones into the maternal and fetal circulations (Cooke et al., 2021).

2a. Placentation in vertebrate evolution

In vertebrate evolution, the origin of a placenta in a clade must be preceded by the transition of its reproductive mode to a viviparous ('live-bearing') condition from an oviparous ('egg-laying') ancestor. Viviparity has independently originated in vertebrates ~150 times (Fig. 1C) (Blackburn, 2015, Griffith and Wagner, 2017). Viviparity may necessitate a change in maternal nutrient provisioning to the fetus, and thus the transition to viviparity is sometimes coupled to a transition to matrotrophy (Blackburn, 1992, Ostrovsky et al., 2016, Whittington et al., 2022b). In contrast to lecithotrophy, where nutrition for the embryo comes in the form of a finite deposited yolk source, matrotrophic organisms continuously provision embryos with nutrients from the mother throughout gestation.

Although many live-bearing vertebrates do not use placentas to facilitate nutrient provisioning ('placentotrophy'), complex nutritive placentas have originated multiple times independently in different viviparous vertebrate clades (Whittington et al., 2022a) (Fig. 1C). Matrotrophy has benefits over lecithotrophy as it permits greater maternal control over nutrient provisioning to the developing fetus, allowing differential investment of

resources depending on maternal capacity (Marsh-Matthews and Deaton, 2006). Matrotrophy comes at the cost of requiring resource sharing across gestation, increasing the potential for materno-fetal conflict (Zeh and Zeh, 2000, Crespi and Semeniuk, 2004, Whittington et al., 2022b).

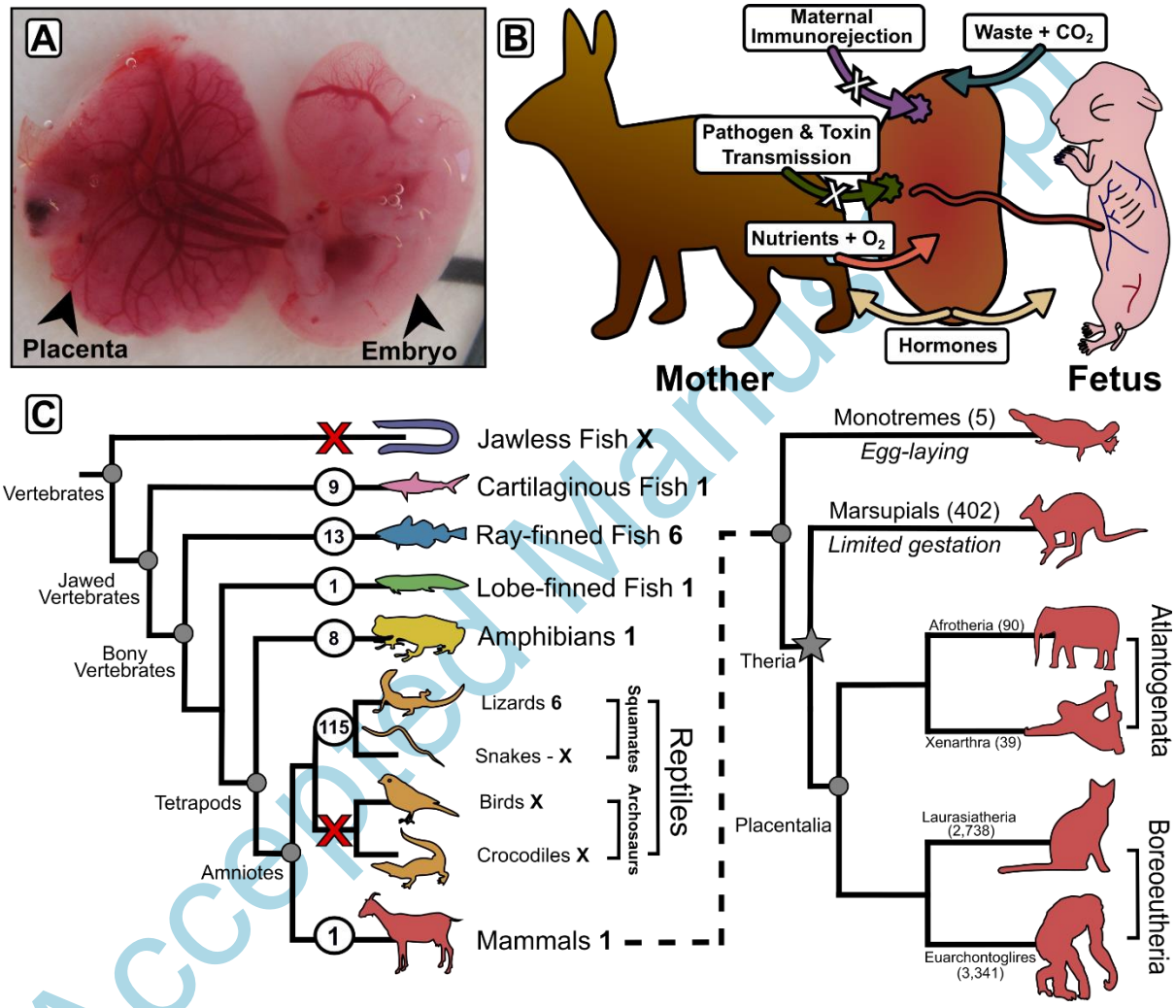

Figure 1 - Placentas have evolved multiple times in vertebrates but only once in mammals. *(A) The placenta is responsible for physiological exchange between mother and fetus during gestation. Pictured is a Day 12 rabbit embryo with attached placenta. (B) Diagrammatic overview of the major functions of the placenta. (C) Number of independent origins of viviparity (white circles) in major vertebrate lineages, as outlined in (Griffith and Wagner, 2017, Blackburn, 2015). Bold numbers next to common names refer to the numbers of independent origins of complex nutritive placentas in each lineage from*

(Whittington et al., 2022a). This strict number excludes non-nutritive placentas involved in gas exchange. An X means that a clade has never evolved viviparity or nutritive placentas. Viviparity evolved only once in mammals (star). Viviparous mammals nourish their young in the womb by a nutritive placenta. Mammalian phylogeny adapted from (Upham et al., 2019). Species numbers of major mammal lineages (brackets) taken from the ASM Mammal Diversity Database https://www.mammaldiversity.org/index.html (Burgin et al., 2018). Animal silhouettes from PhyloPic https://www.phylopic.org/.

2b. Placentation in mammal evolution

Viviparity evolved only once during the evolution of the mammals (Fig. 1C). The mammals consist of three major groups. The monotremes (platypuses and echidnas) are exclusively egg-laying mammals and do not bear live young. In contrast, reproduction in marsupials (kangaroos, opossums, and kin) is viviparous. Although marsupials develop a simple placenta derived from the yolk-sac during a very short gestational period, neonates are born immature and maternal lactation dominates as the major form of nutrient provisioning. This contrasts with the Placentalia, or 'placental mammals', where the definitive placenta (derived from the chorion and allantois, usually after a temporary yolk sac placenta) controls nutrient provisioning across a longer gestation and lactation is secondary relative to marsupials. We will henceforth limit our discussion to placentas from placental mammals. The placenta found in mammals which have one is descended from a common ancestral placenta that evolved ~130 million years ago (Griffith and Wagner, 2017); however, placentas in placental mammals are radically structurally diverse. These structural variations have been grouped into a hierarchical set of divisions used to categorise placentas from placental mammals into a qualitative schema of so-called 'placental types' (Fig. 2-3) (Wildman et al., 2006, Furukawa et al., 2014, Burton, 2022).

Currently, placentas in placental mammals are categorised at three scales: gross morphology, materno-fetal tissue interdigitation, and number of tissue layers (Wildman et al., 2006, Furukawa et al., 2014, Burton, 2022). Gross morphology refers to the general shape of the placenta and specifically to how much of the uterine surface is apposed to

the placenta. The major categories of gross morphology are, from lowest relative placental coverage of the womb's surface area to highest: discoid (e.g. humans, rodents, and bats), zonary (e.g. elephants and carnivores), cotyledonary (e.g. ruminants), and diffuse (e.g. horses, pigs, and whales). The next level of placental organization refers to the degree to which maternal and fetal tissues interdigitate, with more intimate organizations providing a higher relative surface area for exchange. From the lowest degree of interdigitation to the highest, five major placental types are recognised: folded (e.g. pigs), lamellar (e.g. carnivores), trabecular (e.g. some monkeys such as marmosets), villous (e.g. humans, horses, and ruminants), and labyrinthine (e.g. rodents, elephants, and manatees).

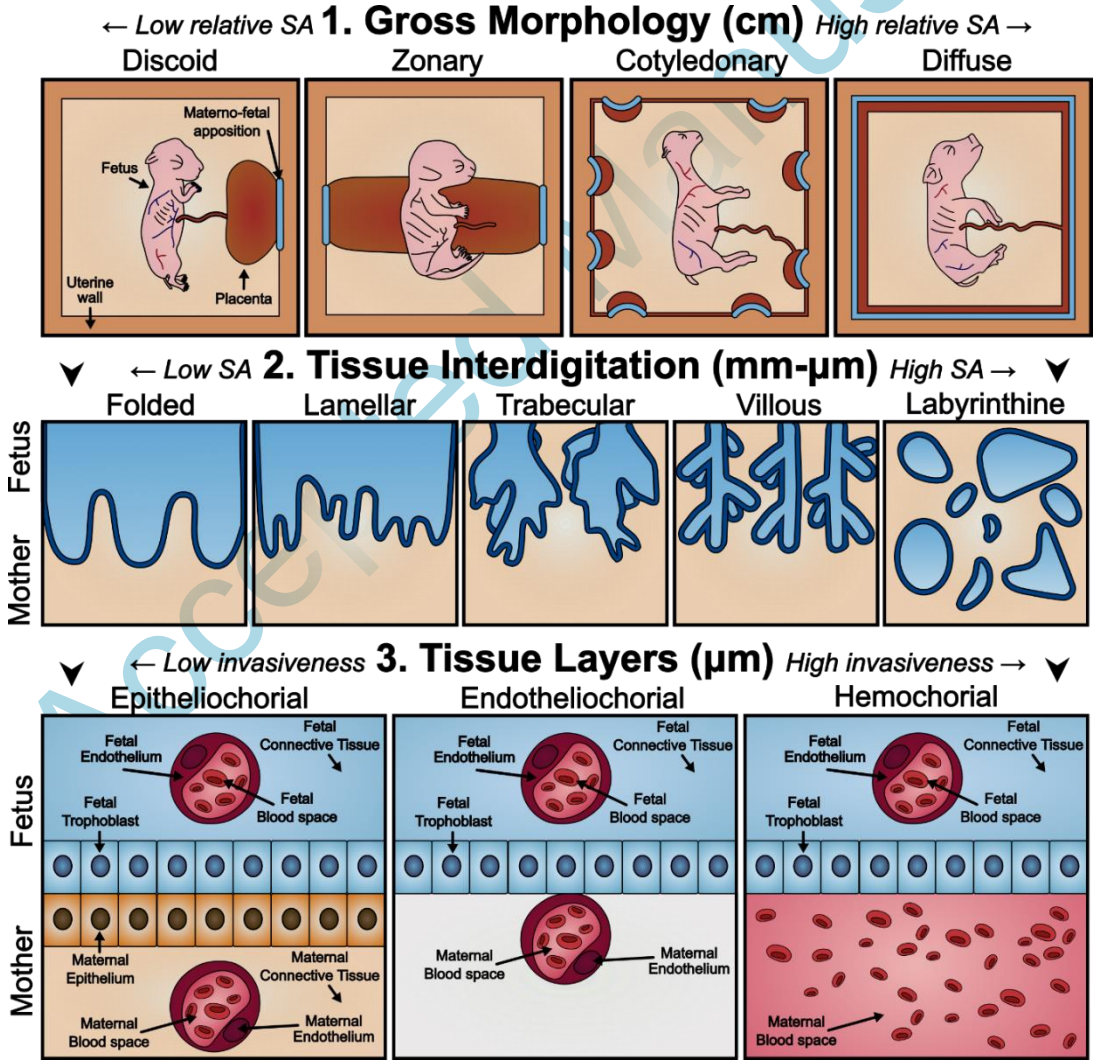

Figure 2 - Placentas from placental mammals are categorised into qualitative structural types. *Diagrammatic overview of the qualitative schema traditionally used to categorise placentas in placental mammals, 1. Gross morphology, 2. Tissue interdigitation and 3. Number of tissue layers (placental invasiveness).*

Finally, the placenta can be characterised by the number of tissue layers separating maternal and fetal circulations. Although three major placental tissue layer types surround fetal circulation (trophoblast, connective tissue, and endothelium), the maternal tissue layers separating maternal blood vary. The most superficial organization is the epitheliochorial condition (e.g. horses and ruminants), where the placenta is in contact with the uterine epithelium. A more intimate arrangement is endotheliochorial organization (e.g. carnivores, some bats, and elephants), where the uterine epithelium and maternal connective tissue recede and the placental trophoblast (the outer layer of cells important for nutrient uptake) is in direct contact with the maternal blood vessel endothelium. Finally, in haemochorial placentas (e.g. humans and rodents), the trophoblast is directly bathed in the mother's blood. Haemochorial placentas are termed 'more invasive' than epitheliochorial or endotheliochorial placental types (Wildman et al., 2006, Furukawa et al., 2014, Burton, 2022).

By using ranked categories of materno-fetal association as proxies for measures such as nutrient transfer efficiency, these types have been used as metrics to explore the drivers of placental evolution. The central premise is that placentas displaying higher levels of materno-fetal intimacy are assumed to permit more efficient nutrient transfer from mother to fetus (efficiency here refers strictly to 'nutrient transfer per volume of placenta per unit time'). Finding clear associations of placental types with different life history strategies or environmental variables has proved difficult, but some correlations have already been identified.

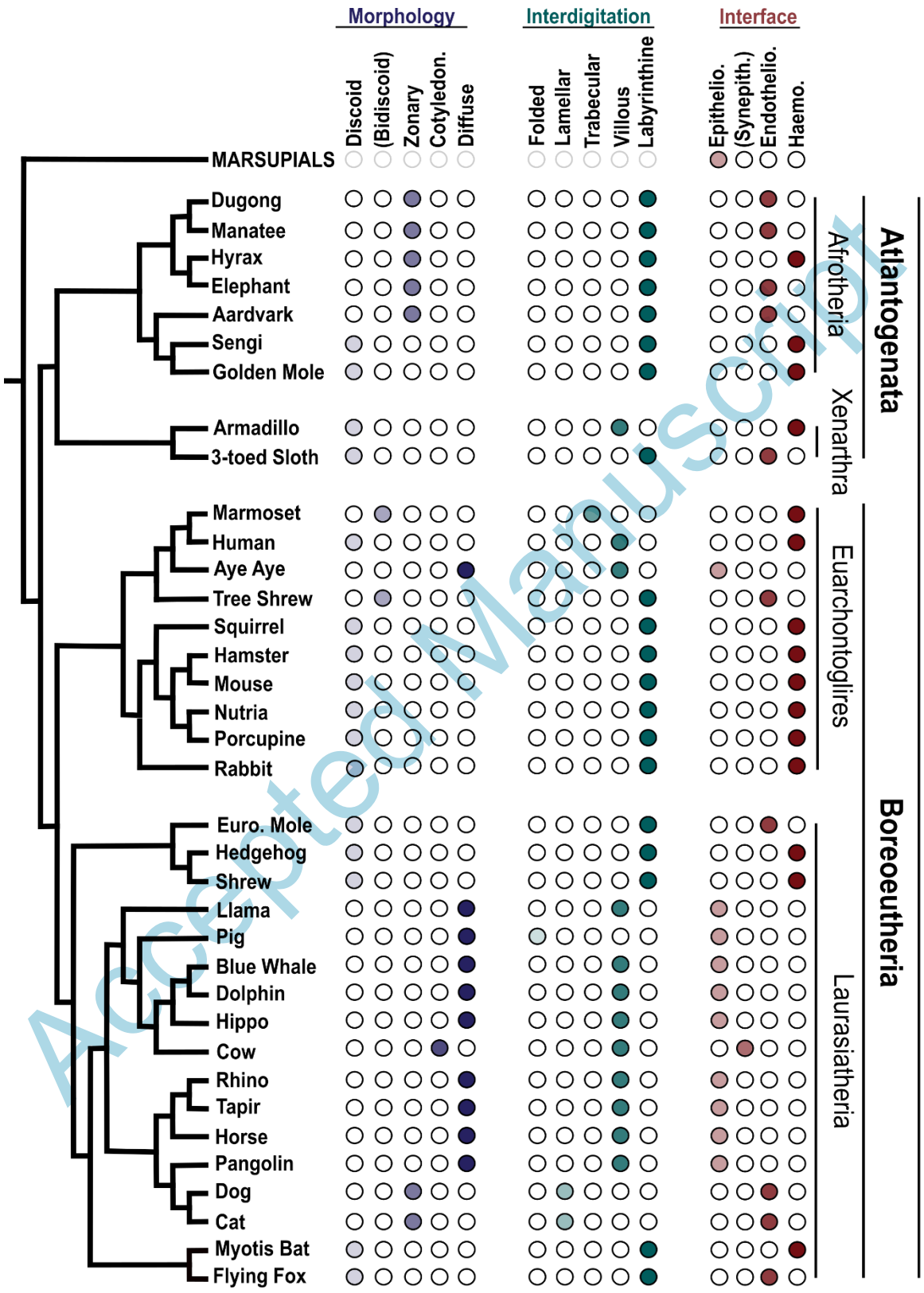

Figure 3 – Phylogenetic distribution of placental type combinations across the Placentalia. *Placental structural traits from the three major traditional categories mapped*

against placental mammal phylogeny using notable representative species as examples. 'Bidiscoid' and 'Synepitheliochorial' are bracketed as, although seen in placental literature, they are functionally similar to discoid and epitheliochorial respectively and are not discussed further here. The marsupial yolk sac placenta is included here as an outgroup to the Placentalia. A more comprehensive list of placental types in placental mammals can be found in (Elliot and Crespi, 2009).

3. HYPOTHESES FOR PLACENTAL STRUCTURAL VARIATION IN PLACENTAL MAMMALS

The concept of placental transfer 'efficiency' is a central assumption to hypothesis generation in placental evolution in placental mammals. Essentially, the concept states that materno-fetal nutrient exchange is easier in some placental types than others. The transfer efficiency of certain species' placentas is derived from examination of placental types, with more interdigitated and more invasive placentas assumed to have a higher transfer efficiency due to the higher relative surface area for materno-fetal exchange and fewer cellular layers acting as barriers to exchange respectively.

3a. The evolution of 'high efficiency' placentas in placental mammals

It has been suggested that more interdigitated and invasive placentas are associated with higher fetal growth rates (Leiser and Kaufmann, 1994, Capellini et al., 2011, Lewitus and Soligo, 2011), and the rate of fetal brain development (Elliot and Crespi, 2008) due to their putative higher efficiency of nutrient transfer; however these proposals are not universally accepted (Martin, 2008). Different placental types have been correlated with life history strategies, with more interdigitated placentas associated with decreased gestation times and large litters of altricial young (Capellini et al., 2011). The inverse is also true, where the independent evolution of villous interdigitation from labyrinthine ancestors is associated with the onset of reproduction later in life, fewer offspring per year, and an increased lifespan (Garratt et al., 2013). Highly interdigitated placentas may enable the evolution of altricial life strategies, perhaps in response to higher maternal mortality or intermittent resource availability (Capellini et al., 2011, Garratt et al., 2013).

Initially, it was proposed that an epitheliochorial placenta represented the ancestral condition in placental mammals (Turner, 1876); however, subsequent character trait mapping against established phylogenies and ancestral trait reconstructions (Vogel, 2005, Wildman et al., 2006, Carter and Mess, 2007, Elliot and Crespi, 2009, Mika et al., 2022) suggest the ancestral placenta in the placental mammals was discoid, labyrinthine, and invasive (either endotheliochorial or haemochorial). It has previously been argued that invasive placentation was only made possible in placental mammals by the mammalian innovation of platelets that permit sufficient postpartum haemostasis and prevent the mother from bleeding excessively after delivery (Martin and Wagner, 2019). Likewise, the transition to more invasive placental types may require molecular prerequisites, such as fusogenic retroviral envelope genes seen in mammals responsible for cell-cell fusion (Lavialle et al., 2013, Funk et al., 2019), illustrating that selective pressures alone cannot drive placental evolution in a vacuum.

3b. The evolution of 'low efficiency' placentas in placental mammals

As the fetal genome is half paternal, the long-term fitness strategies of mother and offspring can come into conflict. This evolutionary arms race is named the 'conflict hypothesis' and some studies suggest that it is a major driver of placental evolution (Haig, 1993, Klisch and Mess, 2007, Wang et al., 2013, Kazemian et al., 2019). For example, it is possible that fetuses with highly efficient placentas could extract excessive nutrients from the mother to the detriment of her own fitness through decreased future reproduction, while benefitting paternal fitness through the increased growth of the fetus. Epitheliochorial placentation in species with precocious, energy intensive young may therefore have evolved to enable greater 'maternal constraint' in nutrient investment for the sake of the mother's future reproduction (Lewis et al., 2012).

Factors other than transfer efficiency have also been proposed as selective pressures favouring 'less efficient' placentation. Some parasites can move from mother to fetus more easily in haemochorial species (Loke, 1982, Webster and Kapel, 2005), and it has been proposed that epitheliochorial placentas evolved in environments with high parasite loads. Capellini and colleagues suggest that this is true for bacteria; however, the opposite pattern was shown for eukaryotic microbes in the same study (Capellini et

al., 2015). Other proposed benefits include a reduced risk of immunorejection of the fetus by the mother (Moffett and Loke, 2006, Roberts et al., 2016), which as an intracorporeal organism with only half of the mother's genome can be considered as a semi-allograft (Hemberger, 2013), and protection of the mother from the oxidative stress generated by fetal metabolism over long gestational periods (Elliot, 2016). These hypotheses highlight how countervailing pressures may select for a placental configuration which, despite having decreased transfer efficiency, is evolutionarily optimal.

4. RECENT DEVELOPMENTS IN 3D PLACENTAL IMAGING

While there has been considerable work in investigating the evolution of the placenta, resolving clear evolutionary drivers of placental structural diversity has proven difficult in the placental mammals. Many factors likely contribute to this, from species sampling biases to reliability in phylogenetic modelling. We propose that another contributing factor is considering placental structures only within the framework of the qualitative schema of placenta types. We hypothesise that grouping placentas into qualitative types for evolutionary comparison misses key variation between species and produces assumptions about transfer efficiency that may obscure the evolutionary picture. Previous studies testing fundamental hypotheses in placental evolution, from the conflict hypothesis to maternal constraint, have hinged on ranked placental types accurately reflecting degrees of transfer efficiency. It is not at all obvious that this is the case, as even the order in which these categories are ranked is not universally agreed upon.

Due to recent advances in 3D imaging techniques, we are now able to image individual placentas across scales to generate high-resolution, quantitative 3D datasets. Although qualitative 3D images of placentas have long been generated in the past by historic techniques such as wax plate reconstruction (Born, 1880, Hill, 1932), modern 3D imaging techniques permit the morphometric quantification of the imaged structure. This enables the full quantification of placental structures in their native 3D, identifies novel structures that may contribute to transfer efficiency, and permits physiological modelling within actual 3D tissue architecture, all of which can assess whether the transfer efficiency of different placental types (and the order in which they are ranked) can be supported.

Quantitative morphometric data can capture more variance and generate higher power for statistical modelling than qualitative categories and can therefore provide greater potential to identify differences and patterns.

Human and mouse placentas have been previously imaged using both transmitted (Haeussner et al., 2014) and fluorescent (Hamid et al., 2005, Mayo et al., 2016, Perazzolo et al., 2017, Mayo et al., 2019) light to reconstruct the 3D structure of villi and vascular trees. 3D X-ray microscopy (microCT) has been used to resolve placental vascular networks from both soft tissue (Aughwane et al., 2019, Tun et al., 2021, Barapatre et al., 2020) and resin casts extracted from tissue impregnation (Junaid et al., 2017). The segmented 3D datasets acquired in the studies above were used for downstream morphometric analysis of placental structures, such as calculating branching hierarchies in villous trees (Haeussner et al., 2014) or quantifying vessel networks in terminal villi (Mayo et al., 2019). A striking application of microCT in mouse placentas is contrast-enhanced microCT (CE-CT), which permits the scanning of tissue incubated in a contrast agent to generate high resolution 3D volumes of whole placentas (De Clercq et al., 2019).

The application of 3D volume electron microscopy (vEM) has been particularly informative in investigations in the human placenta, revealing novel nanoscale structures not easily discernible with lower resolution methods (Lewis, 2023) (Fig. 4A). Important in the context of evolutionary comparative placentation are nanoscale structures which could contribute towards materno-fetal exchange efficiency, such as those that alter diffusion distances or surface area. These include the discovery that infolding of the syncytiotrophoblast (a syncytial organisation of the trophoblast found in humans and some other species) basal membrane increases available surface area ~4.4x more than previously appreciated (Fig. 4F) (Tashev et al., 2022). A noteworthy example is also observed in the recent discovery of trans-syncytial nanopores (TSNs), nanoscale channels that puncture completely through the syncytiotrophoblast and may represent a novel route for diffusion between cells from mother to fetus (Fig. 4G) (Lewis et al., 2022). TSNs also emphasise the importance of specifically applying 3D imaging, as any 2D image of these nanopores simply resembles a vesicle (Fig. 4H) and the apical to basal connectivity can only be resolved in 3D (Fig. 4I).

A fascinating example of vEM within the context of evolutionary placentation was used to explore the conflict hypothesis. Kazemian and colleagues used vEM to reconstruct both maternal and fetal microvilli of different ungulate species with epitheliochorial villous/folded placentas (Kazemian et al., 2019). The conflict hypothesis would predict that, as sites of active transport, fetal microvillous surface area should be higher than maternal as the fetus strives to extract more nutrients from the mother than it is optimal for her to give. This was found to be the case.

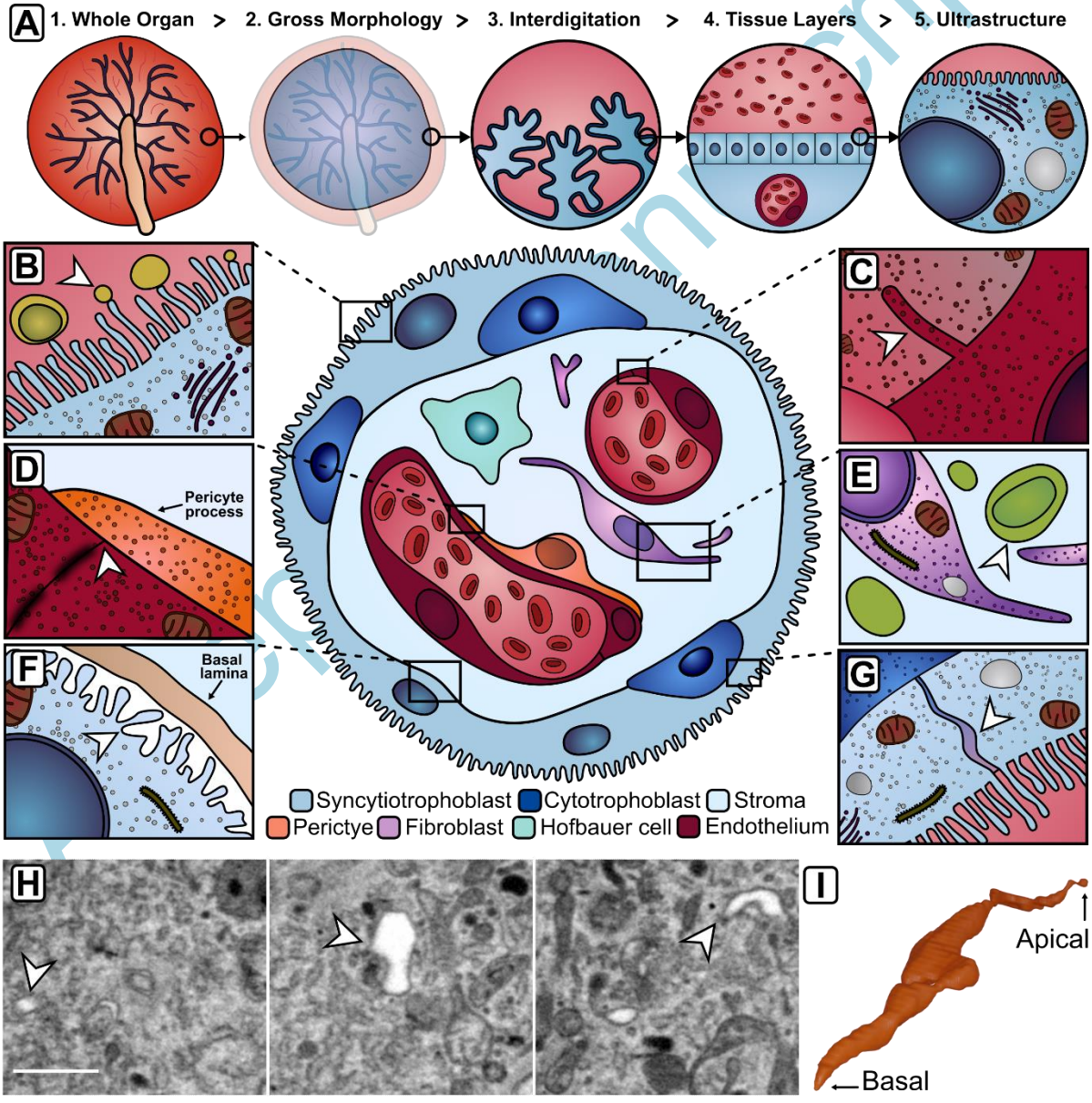

Figure 4 - 3D nanoscale imaging has changed the way we understand the organisation of the human placenta. *(A) In addition to the existing three structural divisions, placentas should also be considered at the ultrastructural and whole organ scale. (B-G) Diagrammatic summary of how recent ultrastructural findings using 3D electron microscopy have updated the traditional understanding of villus organisation in the human placenta (centre). Insets show microvesicles on the tips of microvilli (B) (Davies et al., 2022), inter-endothelial cell processes (C) (Palaiologou et al., 2020b), association of pericyte processes and endothelial junctions (D) (Harris et al., 2021), stromal macrovesicles (E) (Palaiologou et al., 2020a), the infolding of the basal membrane in the syncytiotrophoblast (F) (Tashev et al., 2022), and a trans-syncytial nanopore (TSN) (G) (Lewis et al., 2022). TSN's are a powerful example of the potential of 3D nanoscale imaging (H-I). In 2D sections, they are indiscriminate inclusions (H); however, when reconstructed in 3D, they represent novel structures connecting the apical and basal surfaces of the syncytiotrophoblast (I). Scale bar = 1 µm. For a detailed description of these structures and cell types, we refer readers to the cited literature.*

5. PLACENTAL EVOLUTION IN 3D: FUTURE DIRECTIONS

High-resolution 3D imaging of placental tissue has the power to quantify tissue architecture in unprecedented detail and identify novel structures; however, imaging alone is insufficient to address the unresolved questions in placental evolution. 3D images must be acquired across structural scales, quantified, and integrated into multiscale physiological models. Only then can the concept of transfer efficiency, or any other morphometric or physiological variable, be examined across the whole organ and used for interspecies comparison. Here, we present a roadmap towards this understanding in which we outline methodological approaches, illustrated with primary examples from our work (Fig. 5), and questions to address.

5a. An experimental roadmap towards resolving placental efficiency in 3D

To address the questions outlined here, we propose that we need quantitative metrics representing the multiscale nature of the placenta. To achieve this, multiscale 3D structural data must first be acquired for each species of investigation; however, relying

on any one 3D biological imaging technique comes with inherent trade-offs, mainly the trade-off between spatial resolution and field of view. In practice, this means that the finer the structure that can be resolved, the lower the biovolume of tissue that can be imaged and vice versa. Correlative imaging workflows (Laundon et al., 2023) permit the application of multiple 3D imaging techniques to the same sample (Fonta and Humbel, 2015) to generate multiscale structural data.

Correlative imaging techniques can be used to link established 2D histology with 3D architecture (Fig. 5C). 3D X-ray histology (XRH) (Katsamenis et al., 2019) involves scanning wax-embedded tissue in 3D with microCT techniques prior to sectioning and staining. The subsequent 2D histology images can then be correlated back into the 3D microCT dataset, and the 3D architecture of stained features elucidated. This is particularly important as interpreting 3D placental structures from 2D histological sections has been shown to be unreliable when grounded against 3D imaging techniques (Haeussner et al., 2015). Immunohistochemistry staining can also be conducted with XRH whereby the localisation of targeted proteins can be related to 3D tissue architecture, linking molecular profiling with structure. Another example of correlative microscopy is correlative X-ray and electron microscopy (CXEM), which non-destructively scans a tissue sample with microCT prior to vEM imaging (Fig. 5D) (Laundon et al., 2023). This delivers both a microscale dataset of the entire sample biovolume and a nanoscale dataset of a subsample of the tissue. A previous concern about these approaches has been the time required for the analysis, but with existing semimanual and AI image analysis approaches it would be entirely feasible to test an evolutionary hypothesis within the scope of a research project timeline.

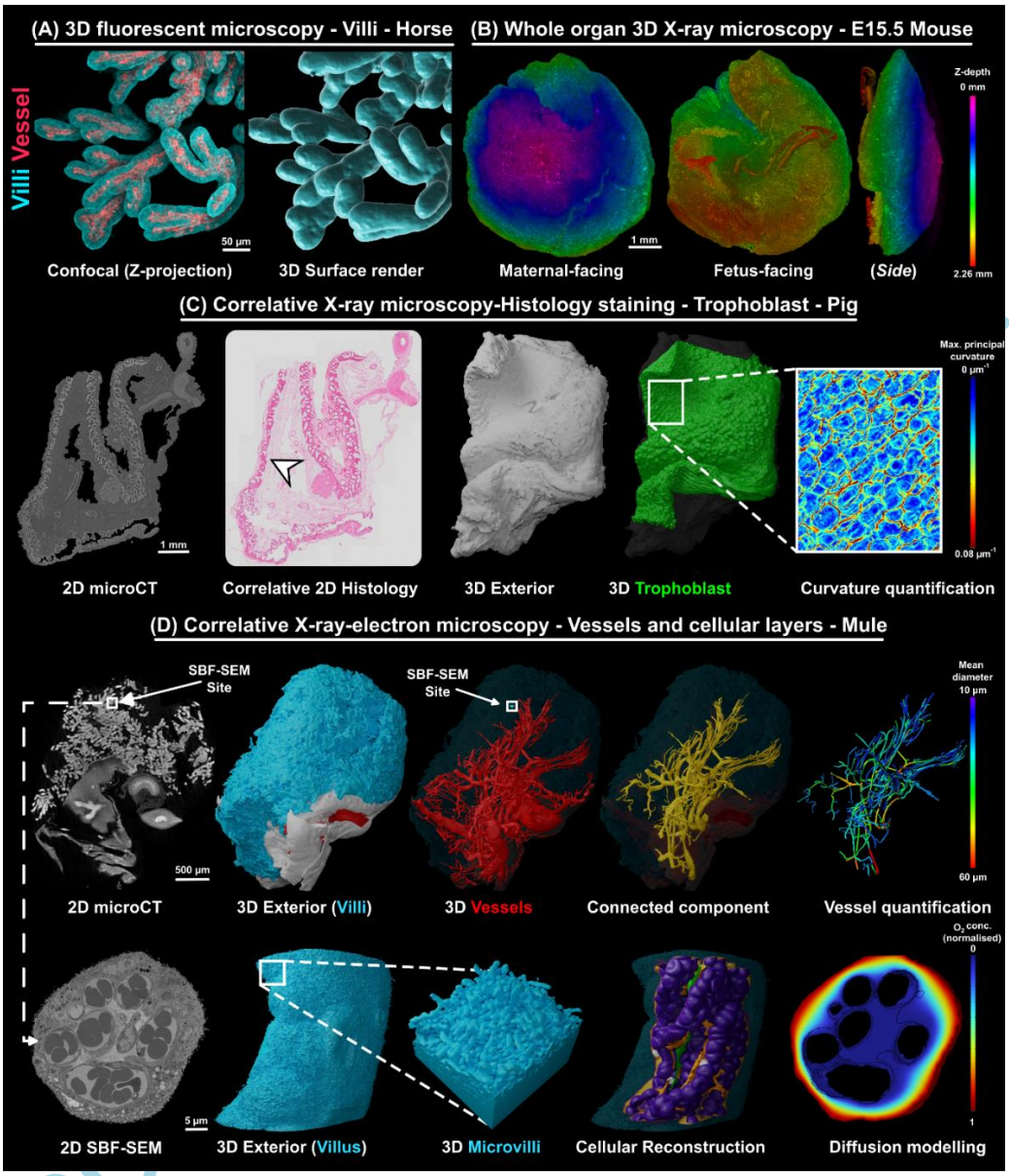

Figure 5 – 3D imaging techniques are powerful tools for quantifying placental structures in placental mammals. *Examples of 3D imaging techniques and quantitative visualisation of non-human placental structures. (A) 3D confocal image and associated surface render of horse placental villi. (B) Whole organ 3D X-ray microscopy (microCT) of an entire Day 15.5 mouse placenta, pseudo-coloured by z-depth. (C) Correlative microCT and 2D histology staining workflow used to identify and 3D reconstruct a section of trophoblast (arrowhead) from a Day 100 pig placenta, pseudo-coloured by surface curvature. (D) Correlative microCT and 3D electron microscopy (SBF-SEM) workflow for multiscale imaging of a section of mule placenta. Top row shows microCT imaging of the*

major blood vessels, and the quantification of the diameters from the largest connected component. Bottom row shows the correlative SBF-SEM imaging of a villus subvolume from the same sample, highlighting the 3D reconstruction of villus cellular layers and the computational modelling of oxygen diffusion in the reconstructed volume.

The aim of 3D imaging of placental structures is to integrate the 3D reconstructions and quantitative morphometrics from these datasets into multiscale computational models that describe the placental organ as a whole. First, it will be necessary to integrate high-resolution nanoscale data from vEM images across larger biovolume datasets (Fig. 6). Such multiscale structural modelling approaches the idea of a placental 'morphome', defined as the total sum of every morphometric component within an organ (Lucocq et al., 2015, Mayhew, 2015).

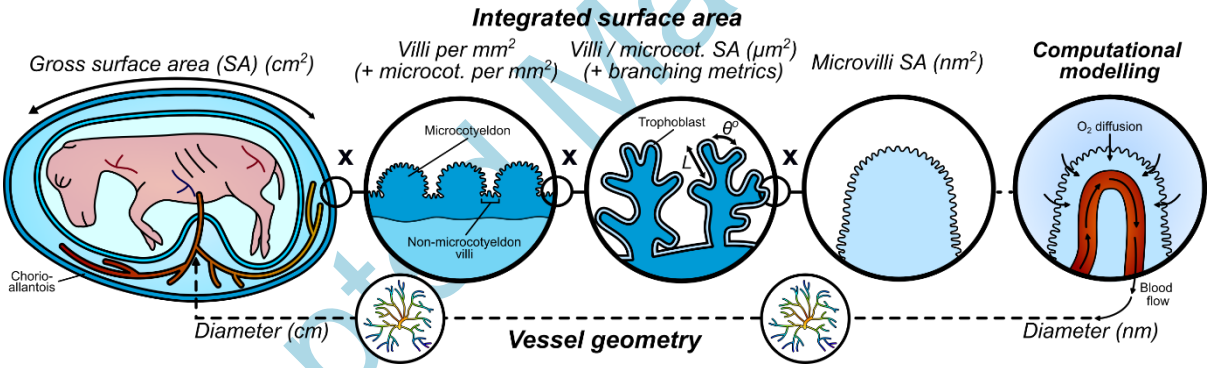

Figure 6 – Towards an integrated multiscale perspective of placental evolution in placental mammals. *Diagrammatic summary of multiscale surface area quantification and transport modelling through the fractal vessel geometry of a horse placenta, as an example for future integrated investigations into placental function and evolution.*

Then, if the hypothesis that different placental types permit different transfer efficiency is to be supported in placental evolution, relative physiological functions must be modelled across placentas from different placental mammals. 3D reconstructed image datasets have previously been used to model physiological functions such as diffusive capacity (Mayo et al., 2016), intervillous flow (Perazzolo et al., 2017, Tun et al., 2021),

and solute diffusion (Lewis et al., 2022) within the boundaries of actual 3D tissue architectures. Multiscale computational models have also been employed using morphometric data in human placentas to model processes such as blood flow (Clark et al., 2015), oxygen transport (Lin et al., 2016), and shear stress on the syncytiotrophoblast (Lee et al., 2023).

When multiscale models are developed within the bounds of placental morphomes, then whole organ function can be modelled and compared between species of placental mammals. These models could also become integrated with multi-omic data, such as spatial transcriptomics and metabolomics, to approach a true 'systems biology' understanding (Kitano, 2002, Mayhew, 2015) of placental evolution. By modelling the different kinds of uptake of substances such as oxygen (passive transfer) (Fig. 5D), amino acids (transporter-mediated transfer), and iron (endocytosis-mediated transfer) (Carter, 2012) through 3D reconstructed tissue geometries, assumptions about certain placental arrangements being more efficient than others can be tested.

5b. Placental evolution from a 3D and multiscale structural perspective in placental mammals

The approaches outlined above will provide quantitative metrics to assess placental efficiency and provide an alternative to the qualitative schema previously used to assume efficiency in placental evolution. If it is true that individual placental types do permit different levels of transfer efficiency, then this finding must also hold true when integrated across scales to consider the whole placenta. If it is established that the overall placental efficiency is similar between species, then framing the evolutionary discussion is terms of efficiency is no longer appropriate. If it is established that overall placental efficiency is lower in some species than others, this would focus the direction of future research into resolving the evolutionary drivers underpinning why this would be. This would be particularly informative within the context of different life history strategies (Capellini et al., 2011, Garratt et al., 2013). If species with large, precocious young do indeed have placentas that are overall less efficient, this could provide evidence supporting 'maternal constraint' as a driver of placental evolution.

As stated above, it has been proposed that the conflict between the long term fitness interests of fetus and mother (the 'conflict hypothesis') is a major driver of placental evolution (Haig, 1993, Klisch and Mess, 2007, Wang et al., 2013, Kazemian et al., 2019). It can be qualitatively observed in placental types that there appears to be an inverse correlation between the relative placental coverage of the womb's surface area ('gross morphology') and interdigitation/invasiveness in placental mammals (Fig. 3). Burton noted that species with folded epitheliochorial placental configuration (the assumed least efficient configuration) such as pigs and some primates always combine this organisation with a diffuse morphology (the largest relative gross morphology) (Burton, 2022) and vice vera. Is the inverse correlation between gross morphology and interdigitation/invasiveness enough to compensate for reduced transfer efficiency at lower structural scales? Does this inverse correlation produce a similar transfer 'capacity' (whole placental transfer per unit of fetal weight per unit time) across placentas from placental mammals? If so, and gross placental coverage of the uterine exchange area increases in response to reduced placental invasiveness, this might provide evidence in favour of the conflict hypothesis (if coupled to additional phylogenetic and experimental investigations).

If overall transfer capacity is not compensated for at the scale of gross morphology, do other factors in some 'low-efficiency' placental organisations, such as nutrient transfer through contact with histotrophic nutrient glands (glands which transfer additional nutrients to the placenta through uterine secretions) (Vogel, 2005), mitigate against structural barriers to transfer? How are specialised placental structures involved in histotrophic nutrient uptake, such as areolae (Enders and Carter, 2006), organised to facilitate this? Additionally, the geometric arrangement of maternal blood flow relative to that of the fetus has been used as a proxy metric for placental transfer efficiency (Carter, 2009, Burton, 2022). Full 3D multiscale reconstructions of placental (and maternal) vascular architecture will allow the modelling of blood flow within realistic tissue geometries to test these assumptions. If compensatory adaptations to 'low-efficiency' placental types are found in integrated multiscale structural investigations and transfer capacities between different placental organisations are similar, this would cast doubt on

the use of qualitative placental types to infer whole organ efficiency and as proxies in correlating placental types with life history strategies.

Syncytial trophoblast interfaces have evolved convergently in placentas from placental mammals (Lavialle et al., 2013), but the reason for this is not clear. Full 3D ultrastructural reconstructions of trophoblasts, which are hypothesised to be central to placental evolution and diversification in placental mammals (Lillegraven, 1975), could shed light on the link between subcellular organisation and function. For example, when coupled to modelling, high-resolution 3D reconstructions of syncytial versus cellular trophoblast layers could be used to test the hypothesis that the syncytiotrophoblast permits more efficient transport of nutrients than individual trophoblast cells (Smith, 2015), due to the absence of additional membrane barriers inhibiting cytoplasmic flow. Syncytial trophoblast interfaces have convergently originated from the capture of fusogenic retroviral proteins that have been proposed to be central in driving the evolution of invasive placentation in placental mammals (Lavialle et al., 2013, Funk et al., 2019, Frost et al., 2023). Therefore, a proper understanding of the structural organisation and function of syncytial trophoblast interfaces could highlight selective pressures favouring the incorporation of retroviral proteins and the evolution of placental invasiveness.

5c. Beyond the placenta of placental mammals

Given the repeated convergent origins of placentation in vertebrates (Fig. 1C), multiscale 3D imaging of the diversity of vertebrate placentas could unveil fundamental principles in convergent organogenesis and the structural underpinnings of the evolution of placentotrophy. An obvious next step is to investigate in squamate reptiles (where viviparity has evolved ~115 times independently and complex nutritive placentas six times) which are used as a group as models to explore the evolution of reproductive strategies (Thompson et al., 2002, Van Dyke et al., 2020, Whittington et al., 2022a). Quantitative structural characterisation of homologous tissue layers (chorion, allantois etc), integrated across scales, between closely related oviparous and viviparous (particularly placentotrophic) taxa within a clade, would shed light on how the same tissues can be structurally co-opted to serve different reproductive strategies. Analogous

investigations in teleost fish would elucidate how placentas have evolved from disparate non-homologous precursory tissues (Griffith and Wagner, 2017). These studies would be particularly informative when coupled to transcriptomic profiling to link genotypic and phenotypic approaches. The repeated and recent independent evolutionary origins of the placenta, and its massive structural diversity, make it an excellent model system to investigate evolutionary organogenesis in general (Griffith and Wagner, 2017).

The applications of addressing evolutionary questions through this lens are wide. For example, multiscale surface areas integrating villi, microvilli, and gross morphology (Fig. 6) could, when coupled to physiological modelling, also be applied to quantify the relative uptake efficiency of animal digestive tracts for interspecies comparison (Helander and Fandriks, 2014, Steinmetz, 2019). Likewise, modelling blood flow through vessel geometries could shed light on phenomenon other than nutrient transport, such as heat dissipation, at the centre of evolutionary hypotheses (Speakman and Krol, 2010, Tattersall et al., 2017). Whatever the specific application, resolving and modelling these structures holistically will generate an integrated understanding of their arrangement and put previous assumptions about their function to the test. Then, broader evolutionary questions, such as those concerning life history strategies or environmental pressures, can be quantitatively addressed.

CONCLUSION

The placenta of placental mammals displays unparalleled structural diversity across scales; however, the evolutionary drivers behind this diversity have proven difficult to identify. We argue that considering placentas from placental mammals as holistic organ systems – integrating 3D multiscale structures, computational modelling, and molecular ‘omics – can greatly contribute to resolving these questions. This is particularly relevant in relation to any assumptions that one placental configuration permits more efficient transfer of nutrients from mother to fetus than another. Moving away from structural reductionism, and towards an appreciation of the system as a whole, is not just necessary for considering the placenta but for investigating the evolution of any given organ.

DATA AVAILABILITY

There are no data associated with this work.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

Conceptualisation: DL, RL; Funding acquisition: RL, NG, PC-P; Writing (original draft): DL, RL; Writing (discussion, revisions, and editing): DL, NG, BS, PC-P, RL.

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